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An epidemiological study of the relations between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy and neuropsychological abnormalities in sheep farmers and dippers

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Abstract

***Objectives*—To investigate the hypothesis that chronic low level exposure to organophosphates (OPs) in sheep dips is related to clinically detectable measures of polyneuropathy.**

***Methods*—The design was a cross sectional exposure-response study of sheep dippers and other non-exposed groups. The study group consisted of 612 sheep dipping farmers, 53 farmers with no sheep dipping experience, and 107 ceramics workers. Retrospective exposure information was obtained by questionnaire based on stable and easily identifiable features of sheep dipping found during the first phase of the study; in particular, estimates of handling concentrate and splashing with dilute dip. Neurological assessments were based on a standard neuropathy symptoms questionnaire, and thermal and vibration quantitative sensory tests.**

***Results*—Adjusted for confounders there was a weak positive association between cumulative exposure to OPs and neurological symptoms, the significance of which was dependent on the inclusion of a few individual workers with extremely high exposure. There was no evidence of an association between cumulative exposure and the thermal or vibration sensory thresholds. However, separating the effects of exposure intensity and duration showed a higher prevalence of symptoms, primarily of a sensory type, among sheep dippers who handled the OP concentrate. There was also evidence that sensory and vibration thresholds were higher among concentrate handlers, the highest exposed group of dippers.**

***Conclusions*—The findings showed a strong association between exposure to OP concentrate and neurological symptoms, but a less consistent association with sensory thresholds. There was only weak evidence of a chronic effect of low dose cumulative exposure to OPs. It is suggested that long term health effects may occur in at least some sheep dippers exposed to OPs over a working life, although the mechanisms are unclear.**

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There is general agreement that long term health effects can follow one or more acute episodes of OP poisoning, and epidemiological studies suggest various of long term consequences of exposure (Rosenstock *et al*,[1] McConnell *et al*,[2] and Steenland *et al*[3]).

Many of the studies published on the effects of long term low level exposure show a correlation between exposure and effect, but results have not been as consistent as those after acute poisoning episodes. For example, Stephens *et al*[4] studied 158 sheep dippers and 155 controls (quarry workers). None of the farmers had experienced acute poisoning. The study involved neuropsychological tests and health questionnaires. The authors found significant differences in attention and speed of information processing in the exposed group. Basic neurological examinations performed on a subgroup of both symptomatic and asymptomatic farmers and controls showed signs of sensory disturbance but little evidence of motor dysfunction in farmers.[5]

Ames *et al*[6] studied 45 asymptomatic agricultural pesticide applicators with at least one documented episode of cholinesterase inhibition. The control group comprised 90 men from the same area, an average of 8.7 years younger, but otherwise well matched. They found no difference between groups in nerve conduction, vibration sensation, motor function, or mood.

A recent report by the Royal Colleges of Physicians and Psychiatrists[7] suggests that reports of chronic low dose effects of OPs are limited by few cases, selection bias, and inadequate controls. It is also considered that some cases may be the result of undocumented episodes of acute exposures. A review by the European Centre for Ecotoxicity and Toxicology of Chemicals[8] concluded that animal experiments confirm acute and protracted effects on cognitive function, but have not shown effects of prolonged low level exposure.

The broad aim of the study as a whole was to investigate whether chronic low level exposure to sheep dip OPs is related to clinically detectable measures of neuropathy. More specifically, the hypothesis under investigation was that repeated exposures to OPs may cause cumulative and irreversible damage to nervous tissue, which eventually becomes clinically detectable.

Methods

STUDY DESIGN

The study was cross sectional, of sheep farmers and other low exposure groups during the winter of 1996–7. For practical reasons it was decided to base the study on two areas of the United Kingdom where there is a relatively high density of sheep farming. The areas chosen were Hereford and Worcester in England, and the Borders, Lothians, and Ayrshire in Scotland.

A sampling frame was constructed from databases of annual census data for farms, maintained by the Ministry of Agriculture, Fisheries, and Food (MAFF) for farms in England and Wales and the Scottish Office for farms in Scotland. Letters of invitation were sent to about 1000 farms, in postal regions within the areas chosen, with the aim of recruiting 600 sheep dippers into the study. The population size was large enough to allow several cases of neuropathy to be identified given a background expected prevalence of 1%. Farmers agreeing to participate were asked for details on farm workers who had worked on the farm within the previous 12 months. These people were also invited to participate. Farms were not included if the farmer had never dipped sheep, or was principally employed in non-farming activities, was retired, single handed, or where there were no suitable facilities for carrying out the survey at the farm.

To augment the number of subjects with low exposure in the study it was intended to recruit a group of about 80 farmers without sheep together with a group of around 120 ceramics workers (brick makers). Pig farm census data were provided by MAFF and the Scottish Office for the same geographical areas covered by the sheep farm data and 55 farmers were recruited in the manner already described. Also, 25 chicken farmers were recruited in Scotland on the basis of local knowledge of the location of farms. The ceramics workers were recruited with the assistance of the British Ceramics Confederation. Two companies were selected and visited, one in south east Scotland and the other in the English midlands.

EXPOSURE ESTIMATION

Retrospective exposure information was obtained for the period of common usage of OP sheep dips (1970 onwards) with an exposure history questionnaire developed during the first phase of the study described in the companion paper.[9] Briefly, the first phase included an occupational hygiene study of dipping practice at 20 farms. A simple empirical dermal exposure model was derived for measured urinary metabolites of OPs, diethylphosphate (DEP), and diethylthiophosphate (DETP), and compared with hygientists' observations of working practice. This model identified the handling of the concentrate dip product (which was usually the responsibility of a single dipper) as the principal source of exposure among dippers. An effect due to splashing with dilute dip in the bath was also found. For each job that involved dipping sheep, questions were asked about: the number of days spent dipping with OP dips, the proportion of dipping days when concentrate was handled, and the proportion of dipping days in each of the three principal tasks (plunger, chucker, and helper). Information was also gathered on the use of gloves and other personal protective equipment while dipping.

Cumulative exposure to OP dips (OPEXP), the sum of the concentration of urinary metabolites of DEP and DETP (in units of nmol/mmol of creatinine) across dipping days, was the weighted sum of cumulative exposure to both concentrate (CONC) and dilute dip splash (SPLASH). CONC was an estimate of the total number of concentrate handling events, SPLASH was a cumulative time weighted splash score based on hygienists' observations of splashing within each of the three dipping tasks. A simpler exposure variable measuring the total number of dipping days (DAYS) was also calculated.

The daily intensity of exposures to both concentrate and splash were estimated by dividing cumulative exposure variable OPEXP, and its components CONC and SPLASH, by the total dipping days (DAYS).

The exposure history questionnaire was administered, by interview, by trained technicians on farm premises. An early version of the exposure history questionnaire was piloted during the first phase of the study and technicians were trained in administering the questionnaire with both mock interviews in house and interviews with local farmers.

NEUROLOGICAL SYMPTOMS AND SENSORY TESTS

Neurological assessments were conducted on farm premises using a symptoms questionnaire in conjunction with a series of quantitative sensory tests based on the Mayo Clinic method.[10] The original questionnaire was designed for clinical use, whereas in this case the questionnaire was administered by a trained technician in the field.

Symptoms were categorised into three groups relating to muscle weakness and sensory symptoms, both in upper and lower limbs and indicative of damage to the peripheral nervous system, and autonomic symptoms—such as sweating, fainting, and impotence. Analysis was based on an overall dichotomous symptom indicator, scoring positive for at least one symptom in either the muscle weakness or sensory symptom groups, or two symptoms in the autonomic group. The downweighting of autonomic symptoms was based on the Mayo Clinic questionnaire and was designed to increase specificity.

Heat and cold threshold values were determined by a microprocessor system through a stimulating probe (thermode).[11] Thermal thresholds were expressed as temperature changes from the basal skin temperature. The amount of current passed gave a measure of the amplitude of the stimulus (either heating or cooling). The skin temperature beneath the stimulator was required to be maintained at a constant predetermined value (normally 34°C ±0.2°C) when not being stimulated. This proved difficult in practice, despite local heating of the limb, due to low ambient temperatures in many farm buildings visited.

The Vibrameter produces a varying amplitude of displacement of the skin which acts as a stimulus to the receptors sensitive to vibration. The procedure followed a standard method of test administration.[12]

In all three tests, higher thresholds represented less sensitivity to the stimuli and therefore evidence of nerve damage. All three sensory tests had shown high sensitivity and specificity in a laboratory with controlled ambient temperature. Compared with clinical results, the sensory tests and questionnaire were found to be sufficiently reliable and reproducible for diagnostic screening in the field (unpublished data).

STATISTICAL METHODS

Linear logistic regression was used to calculate odds ratios (ORs) for reporting symptoms and to test the significance of the differences between occupational groups and the effect of cumulative exposure.[14] By fitting models that included the relevant variables, ORs were adjusted for potential confounders of neurological symptoms—such as age and alcohol habit. The three sensory test thresholds were compared graphically across the age range with scatter plots that included a locally weighted scatter plot smoother, or LOWESS,[15] to aid the comparison of trends. Multiple linear regression was used to investigate exposure-response relations among sensory test thresholds in the presence of potential confounding variables.

Cumulative exposure variables were scaled in the regression analyses by their interquartile range across all subjects. Unless otherwise stated, a 5% level was used to determine significance, although p values for individual tests lying between 5% and 10% were not ignored but noted to be of borderline significance.

To check the linearity of the exposure-response relations, non-parametric smoothing splines were used in place of simple linear terms for exposure, a feature of generalised additive models.[16] Residual plots were used to investigate the goodness of fit of the models and leverage statistics[14] were used to identify potentially influential observations. Regression models were fitted with Genstat 5, Release 4.1 (Genstat 5 Committee, 1993).[17]

Results

RECRUITMENT

Of the 995 sheep farm owners invited, 611 (61%) initially agreed to participate in the study. The most common reasons cited for non-participation were that the farmer was not interested, or was too busy (22%). About 17% were not followed up either because they did not meet the criteria for inclusion, or because the quota of farms had been reached and the field survey ended before responses could be processed. About half of the exclusions were because the farm personnel had themselves never dipped sheep.

Of the 508 farms followed up, 335 were deemed suitable for survey, with a survey taking place in 293 (88%). The most common reason for surveys not taking place was that, despite the farmer's acceptance, the farm workers were unwilling to take part.

Of the 56 pig farms contacted, it was decided that 35 (62%) were suitable for survey, and all of these farms were in fact surveyed. The remaining 21 farms proved unsuitable for survey, as many had no relevant livestock, or were small holdings, or the owner worked full time at another job.

The Scottish ceramics company employed less than 60 people. Participation rates of over 80% were achieved at this site. The company in the English midlands employed about 150 people, and surveying stopped when about 60% of employees had participated.

CHARACTERISTICS OF THE STUDY GROUP

After exclusion of subjects with a disease or taking a medication that may confound a diagnosis of neuropathy (n=31), or ceramics factory workers with experience of sheep dipping (n=7), the study group for analysis consisted of 772 subjects.

During the survey it was noted that many pig and chicken farmers also kept sheep and so it was decided to make the distinction only between farmers and farmworkers who had dipped sheep (sheep dippers), and those who had not (farmers who were not sheep dippers). Therefore, the study group consisted of 612 sheep dippers, 53 farmers who were not sheep dippers, and 107 ceramics factory workers. Table 1 shows a summary of the characteristics of the study group according to these occupational groups. Sheep dippers were, on average 6 years older than the other groups and consisted of a higher proportion of women (14%). There was little difference in reported alcohol consumption and educational qualifications between sheep dippers and other farmers, although ceramics workers reported greater alcohol consumption than the groups of farmers.

NEUROLOGICAL SYMPTOMS AND SENSORY TESTS

Within all occupational groups autonomic symptoms were most often reported, followed by sensory symptoms, then muscle weakness symptoms (table 2). Based on the overall symptom indicator, sheep dippers reported symptoms more often (19%) than farmers who were not sheep dippers (11%), and ceramics workers (5%). Symptom reporting generally increased with age, particularly after age 55 years, but within age groups, sheep dippers reported all types of symptoms most often.

Despite wide variation between individual workers, all three sensory thresholds showed a positive gradient, indicating decreased sensitivity, with age, that for hot and vibration thresholds was more marked after the age of 45 years (fig 1). LOWESS smoothers were fitted to aid comparison of mean thresholds between the

Table 1 Summary statistics for study subjects

Variable	Ceramics workers (n=107) n	(%)	Farmers not sheep dippers (n=53) n	(%)	Sheep dippers (n=612) n	(%)
Country of residence (n (%)):						
Scotland	36	34	46	87	344	56
England	71	66	7	13	268	44
Age (y):						
15–24	11	10.3	9	17.0	35	5.7
25–34	30	28.0	15	28.3	131	21.4
35–44	40	37.4	7	13.2	142	23.2
45–54	19	17.8	17	32.1	149	24.3
55–64	7	6.5	5	9.4	113	18.5
65–74	0	0.0	0	0.0	37	6.0
⩾75	0	0.0	0	0.0	5	0.8
Mean (SD)	38.6 (10.2)		39.2 (12.7)		45.1 (13.4)	
Sex (n (%)):						
Male	104	97.2	50	94.3	524	85.6
Female	3	2.8	3	5.7	88	14.4
Alcohol (units/week, (n (%))):						
Non-drinker	2	1.9	3	5.7	41	6.7
⩽5	16	15.0	22	41.5	293	47.9
6–15	35	32.7	16	30.2	176	28.8
16–30	34	31.8	10	18.9	78	12.7
31–45	14	13.1	1	1.9	19	3.1
⩾45	6	5.6	1	1.9	5	0.8
Education (highest level (n (%))):						
No certificates	46	43.0	19	35.8	226	36.9
O level/A level*	36	33.7	16	30.2	162	26.5
Tertiary	25	23.5	18	34.0	224	36.6

*Includes standard/higher grade equivalents for Scotland.

groups. It can be seen that, correcting for age, there was no evidence of differences in mean hot and vibration thresholds among the groups. However, there was evidence that cold thresholds among sheep dippers were consistently higher across ages than among farmers who were not sheep dippers. This was also true in comparison with ceramics workers under the age of 40.

EXPOSURE-RESPONSE ANALYSES

Without adjusting for covariates there were significant positive linear effects of both OPEXP and DAYS for symptoms (table 3). A series of linear logistic models were fitted to assess the relation between cumulative exposure and the overall symptoms indicator, allowing for important covariates. The final model included both age (OR 1.42 x10^{-1} years, 95% confidence interval (95% CI) 1.22 to 1.66) and country (OR 1.93 for England *v* Scotland, 95% CI 1.27 to 2.92). Current alcohol consumption was not significantly associated with symptoms. Adjusted for these confounders, both cumulative exposure indices showed a significant positive linear effect for symptoms, but not for the sensory tests (table 3). Total days dipped accounted for the largest improvement in model fit as measured by the decrease in residual deviance. The magnitude of the effect of DAYS was equivalent to a 13% increase in the prevalence odds of symptoms for every 74 days of dipping.

Although after adjusting for age, country, and cumulative exposure (DAYS) there was no mean difference in symptoms prevalence between sheep dippers and farmers who were not sheep dippers, prevalence of symptoms remained significantly higher among sheep dippers than ceramics workers (OR 3.85; 95% CI 1.51 to 9.82).

Multiple linear regression was used to model the sensory test thresholds on the logarithmic scale. Unadjusted for covariates there were relatively weak positive linear effects of the cumulative exposure indices and both cold and vibration thresholds (table 3). However, age was a significant confounding variable for all three thresholds. Due to the possible non-linearity of this effect across all ages, a non-parametric cubic spline term was fitted for age (with smoothness constrained to four degrees of freedom). However, assuming approximate linearity, multiplicative effects ranged from ×1.30 (95% CI 1.24 to 1.36) for the cold threshold, to ×1.64 (95% CI 1.54 to 1.75) for the hot threshold. A term for sex was also included in the threshold models. For all three sensory tests men had higher thresholds than women, with multiplicative effects ranging from ×1.21 (95% CI 0.97 to 1.50) for vibration to ×2.15 (95% CI 1.67 to 2.78) for hot thresholds. Cold thresholds remained significantly higher on average among sheep dippers than farmers who were not sheep dippers and ceramics workers even after adjustment for cumulative exposure.

Adjusting for age, sex, and country effects, there was no evidence of a significant positive association between either of the two principal cumulative exposure indices and the three sensory thresholds (table 3). There was a small, but significant, negative association between cumulative exposure and hot thresholds, which was equivalent to a 6% decrease in hot sensory threshold per 74 days dipped.

The linearity of the estimated cumulative exposure effects was investigated by replacing the simple linear term for DAYS with a cubic smoothing spline (fig 2). In this way, the linear effects in table 3 were confirmed as accurately reflecting the exposure-response trends across most of the distribution of exposures in the study group (<400 days), including the significant effect for symptoms. However, due to the highly skewed distribution of cumulative exposure, a few subjects were highly influential in the fitted exposure-response models.

Table 3 shows the effect on the estimated cumulative exposure gradients of omitting the subjects with highest leverage (>0.05). In the

Table 2 Prevalence of reported symptoms by age and occupational group

Symptom group	Age (y) <35	35–44	45–54	55+	All
Muscle weakness (%):					
Ceramics	0.0	0.0	0.0	0.0	0.0
Farmers not sheep dippers	4.2	0.0	0.0	0.0	1.9
Sheep dippers	3.0	7.0	7.4	14.2	7.8
Sensory (%):					
Ceramics	0.0	2.5	0.0	14.3	1.9
Farmers not sheep dippers	0.0	14.3	5.9	20.0	5.7
Sheep dippers	5.4	11.3	11.4	18.7	11.6
Autonomic (%):					
Ceramics	17.1	5.0	5.3	14.3	10.3
Farmers not sheep dippers	25.0	28.6	17.6	0.0	20.8
Sheep dippers	26.5	24.6	26.8	35.5	28.4
Symptoms indicator (%):*					
Ceramics	4.9	5.0	0.0	14.3	4.7
Farmers not sheep dippers	12.5	14.3	5.9	20.0	11.3
Sheep dippers	9.0	16.9	20.1	31.0	19.1
Age distribution:†					
Ceramics	41	40	19	7	107
Farmers not sheep dippers	24	7	17	5	53
Sheep dippers	166	142	149	155	612

*At least one positive muscle weakness or sensory symptom, or two autonomic symptoms.
†Denominators for prevalence of symptoms.

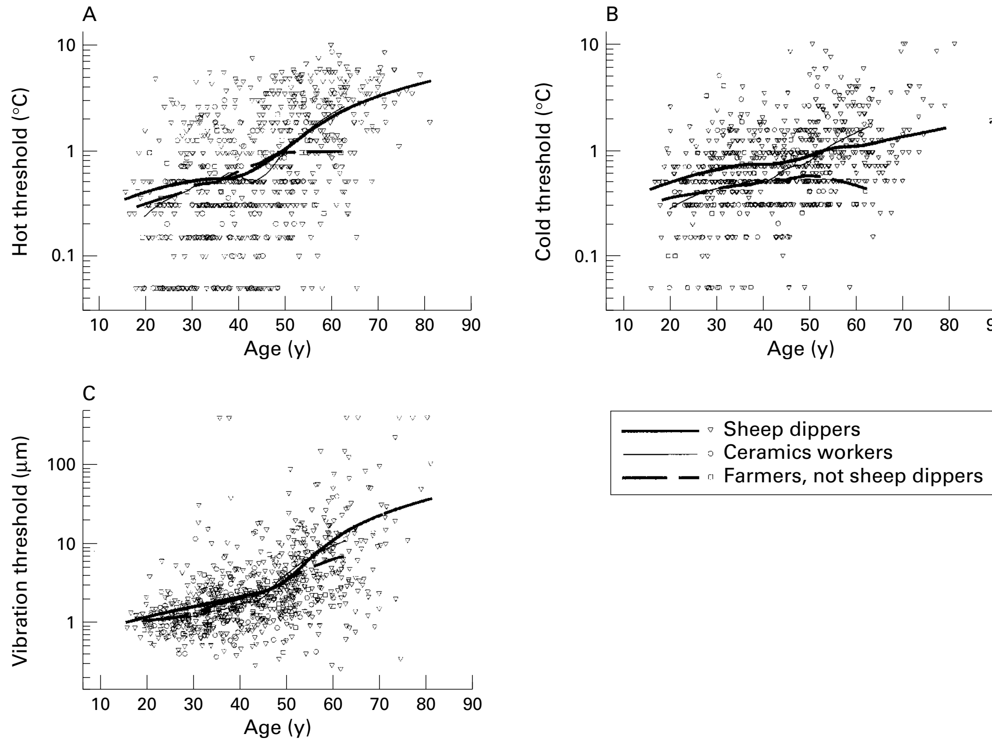

Figure 1 Sensory test thresholds against age for (A) hot sensation, (B) cold sensation, and (C) vibration. Graphs show individual threshold values and LOWESS smoothed trend by occupational group.

logistic regression of symptoms, the four highest leverage points also corresponded to the four highest exposed subjects (DAYS >800), two of whom reported symptoms. Omission of these subjects resulted in a lower OR (1.07) for DAYS, now non-significant, with 95% CI 0.92 to 1.24, and an almost identical result for OPEXP. Thus the significance of the exposure-response gradients for symptoms depended on the inclusion of a very few highly exposed dippers.

Omission of the high leverage points in the multiple regressions of the sensory thresholds had minimum impact, with the significant negative association between cumulative exposure and hot threshold found from the full data set remaining.

EFFECT OF INTENSITY OF CONCENTRATE HANDLING

The effect of average intensity of exposure, as well as duration of exposure, was investigated by adding each of the exposure intensity variables grouping mean exposure intensity to concentrate, splash, and both into quartiles (AVCONC, AVSPLASH, and AVOPEXP, respectively), and adding to the exposure-response regression models that also included the important confounders and variable DAYS (table 4). For symptoms and vibration threshold, concentrate handling intensity was significant (p=0.005), but total intensity of exposure to OP was of only borderline significance (p=0.09). For none of the four response variables was the intensity of splashing significant. This indicated that, as well as duration of exposure, average intensity of exposure to handling concentrate in particular was an important predictor of neurological symptoms. There was no significant interaction between the variables of exposure intensity and duration of exposure, so mean intensity of handling concentrate was assumed to be independent of the total number of days dipped in relation to prevalence of symptoms.

Table 3 Estimates of odds ratios (ORs) (95% CIs) and mutliplicative effects (×) for cumulative exposure variables adjusted for important covariates

Exposure	*Symptoms† (OR)*	*Hot QST‡ (× effect)*	*Cold QST‡ (× effect)*	*Vibration QST‡ (× effect)*
Unadjusted for covariates:				
DAYS	*1.15 (1.04 to 1.26)	0.99 (0.93 to 1.05)	* 1.04 (1.00 to1.09)	*1.06 (1.01 to 1.12)
OPEXP	*1.13 (1.03 to 1.24)	1.01 (0.95 to 1.07)	*1.04 (1.01 to 1.09)	*1.06 (1.01 to 1.12)
Adjusted for covariates:				
DAYS	*1.13 (1.01 to 1.25)	*0.94 (0.89 to 1.00)	0.99 (0.95 to 1.03)	1.01 (0.96 to 1.06)
OPEXP	*1.11 (1.01 to 1.23)	0.96 (0.91 to 1.01)	0.99 (0.96 to 1.03)	1.01 (0.96 to 1.05)
Adjusted for covariates excluding high leverage points:§				
DAYS	1.07 (0.92 to 1.24)	*0.92 (0.86 to 0.99)	0.98 (0.94 to 1.03)	1.03 (0.97 to 1.09)
OPEXP	1.06 (0.91 to 1.24)	*0.93 (0.86 to 1.00)	0.98 (0.93 to 1.03)	1.04 (0.97 to 1.10)

*p<0.05.
†Adjusted for age, country, and occupational group.
‡Adjusted for age, sex, country, and occupational group.
§Omitting 4, 17, 10, and 18 data points for symptoms, hot, cold, and vibration QSTs respectively.
Variables DAYS and OPEXP represent cumulative exposure indices for exposure to OPs and days dipped, scaled by the interquartile range of the values across all subjects: 74 days for DAYS; and 2350 nmol.mmol^{-1}.days for OPEXP.

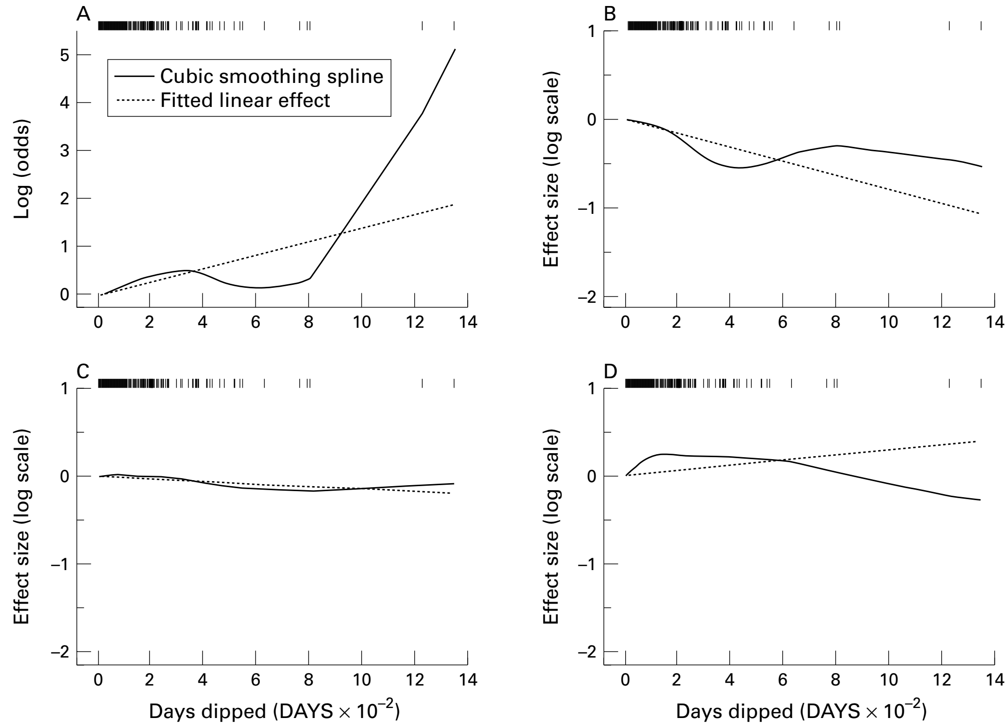

Figure 2 Estimated regression effect of cumulative days dipped, relative to zero days, on (A) prevalence of symptoms, (B) hot sensory thresholds, (C) cold sensory thresholds, and (D) vibration thresholds. Graphs show both linear and non-parametric cubic spline effects, adjusted for age, sex, and country, and show the distribution of days dipped along the top axis.

Further analysis of the shape of the relation between mean intensity of exposure to concentrate and symptoms with a non-parametric cubic spline showed that the nature of the effect was essentially a distinction between those who had, and those who had not, ever been principal concentrate handlers.

Table 5 shows the estimated effects from a linear logistic regression model of symptoms that includes the effect of concentrate handling intensity (ever versus never), and total days dipped as well as important covariates age, sex, country, and occupational group. Principal handlers of concentrate reported symptoms considerably more often than non-handlers (OR 3.4; 95%CI 1.63 to 7.23). As for the models fitted from purely cumulative exposure indices, there were significant effects of age and country. However, men reported symptoms less often than women of the same age, country, and exposure (OR 0.34; 95% CI 0.17 to 0.70). This effect had been masked before adjustment for intensity of handling concentrate because a higher proportion of men (87%) than women (25%) reported having been concentrate handlers. Adjusting for handling concentrate also explained the difference between sheep dippers with low exposure and

Table 4 Significance (p values) of grouped mean exposure intensity variables after adjusting for important covariates and total days dipped

Average exposure intensity	*Symptoms**	*Hot QST†*	*Cold QST†*	*Vibration QST†*
AVCONC	0.005	0.819	0.594	0.031
AVSPLASH	0.509	0.710	0.130	0.276
AVOPEXP	0.093	0.714	0.166	0.067

*Adjusted for age, country, occupational group, and total days dipped.
†Adjusted for age, sex, country, occupational group, and total days dipped.
Variables AVCONC, AVSPLASH, and AVOPEXP correspond with average exposure intensity to concentrate, splash, and both respectively.

Table 5 Estimates of odds ratios (ORs) (95% CIs) and multiplicative effects (×) for handling concentrate (ever versus never, adjusted for important covariates and total days dipped

	Symptoms		*Hot QST*		*Cold QST*		*Vibration QST*	
Terms	*OR*	*CI*	×	*CI*	×	*CI*	×	*CI*
Age:								
$\times 10^{-1}$ y	1.43	(1.22 to 1.67)	1.64	(1.63 to 1.66)	1.29	(1.28 to 1.29)	1.62	(1.61 to 1.63)
Country:								
England *v* Scotland	1.98	(1.30 to 3.03)	1.21	(1.02 to 1.45)	1.11	(0.98 to 1.25)	1.00	(0.86 to 1.16)
Sheep dippers *v*:								
Farmers not sheep dippers	0.37	(0.12 to 1.15)	1.08	(0.73 to 1.61)	1.41	(1.07 to 1.86)	1.06	(0.75 to 1.49)
Ceramics workers	1.27	(0.40 to 4.00)	1.47	(1.05 to 2.04)	1.22	(0.97 to 1.54)	1.19	(0.89 to 1.57)
DAYS:*								
$\times 74^{-1}$ days	1.10	(0.99 to 1.23)	0.93	(0.88 to 0.99)	0.98	(0.95 to 1.02)	0.99	(0.95 to 1.04)
Sex:								
Male *v* female	0.34	(0.17 to 0.70)	2.20	(1.64 to 2.97)	1.29	(1.05 to 1.58)	1.26	(0.98 to 1.63)
Handling concentrate:								
Ever *v* never	3.43	(1.63 to 7.23)	0.92	(0.71 to 1.21)	1.20	(1.00 to 1.45)	1.09	(0.87 to 1.37)

*Total days dipped.

ceramics workers reported earlier, but indicated that, having taken account of differences due to the effect exposure to OP, sheep dippers were less likely to report symptoms than farmers who were not sheep dippers (OR 0.37; 95% CI 0.12 to 1.15).

Table 5 also shows the same models fitted to the three sensory test thresholds. Adjusted for important covariates and total days dipped, those who had acted as concentrate handlers had 20% higher cold thresholds and this result was significant (p=0.05). Adjusted for covariates cold thresholds among sheep dippers who had not handled concentrate remained 41% higher than among farmers who were not sheep dippers.

PROTECTIVE CLOTHING

Almost all dippers reported wearing waterproof footwear on all occasions when dipping. Waterproof trousers were on average worn on more than 80% of dipping days. However, among those who handled concentrate, protective gloves were reported to have been worn on only in a few occasions, as found in the first phase of the study (companion paper).[9] Use of protective gloves rose from about 30% among those with very low intensity of exposure to concentrate, up to almost 50% among those who handled concentrate most often. It was not possible to take account of protective clothing in the exposure-response analyses. The suitability and effectiveness of the clothing is important and it had not been possible to model the effect in the earlier hygiene study of dippers.

OTHER SOURCES OF EXPOSURE

The neuropathy symptoms questionnaire included questions about current exposure to vibration, both occupationally due to use of power tools and machinery and at leisure, through motor cycling, wood working, and vehicle maintenance. Exposure to vibration occupationally and at leisure was minimal and comparable between the groups except for tractor driving. Tractors were driven daily by 77% of sheep dippers for an average of 3 hours a day compared with an average of 2 hours a day among 36% of farmers who were not sheep dippers. Most sheep dippers (71%) used sheep dip products other than during sheep dipping—for example, pour on products— and more had treated cattle for warble fly (63%) compared with farmers who were not sheep dippers (17%). Thirty seven per cent of sheep dippers currently worked with solvents—for example, thinners, greasers, varnishes— compared with 58% of farmers who were not sheep dippers and 44% of ceramics workers.

Each of these exposure variables were added separately to the symptoms regression model in table 5 to check for a confounding effect on the relation with handling concentrate. However, handling concentrate remained significant on each occasion.

Discussion

After adjustment for confounders, there seemed to be a significant effect of cumulative exposure. However, the magnitude of the effect was relatively low compared with the effect of age and the unforeseen effects of sex and country. For example, assuming a working lifetime for a sheep farmer that involved dipping twice a year, for 3 days at a time, over 40 years, the exposure-response model in table 3 would predict a 6 fold increase in the prevalence odds of symptoms. Of this increase however, over two thirds would be due solely to the effects of aging regardless of any effect of exposure to OPs. Equally, although the magnitude of the cumulative exposure effect was comparable both within the two countries and within each sex (data not shown), it was small in comparison with the effects due to differences between country and sex among subjects with the same exposure. As well as the relatively low magnitude of the cumulative effect of exposure relative to reporting symptoms, the significance depended on the inclusion of the four highest exposed subjects (two of whom reported symptoms). Significance could not be shown among the remaining, overwhelming majority, of dippers.

In a sufficiently powerful and reliable study, we would expect to find similar relations among the quantitative sensory test thresholds as were found for symptoms, on the grounds that, biologically, sensory threshold effects should be detectable before the subject is aware of sensory symptoms. Although unadjusted, thresholds were higher among sheep dippers than among the non-exposed groups, when adjusted for age and sex, only for the cold threshold was there evidence that thresholds remained higher among sheep dippers. The inconsistent differences in hot and vibration thresholds among the occupational groups in the two regions surveyed, although not easily explained in terms of possible exposure effects, do not point to an important effect among sheep dippers relative to the non-exposed groups. There was no evidence in general of a cumulative exposure effect on any of the three quantitative sensory test thresholds.

There is likely to have been substantial error in exposure estimates, both from recall of dipping days and tasks and error due to empirical estimates of parameters in the exposure model.[9] This may explain the marginally stronger effect of the simple exposure variable based on recall of DAYS than the variable that also incorporated information on likely OPEXP, which also relied on recall of dipping tasks and the empirical exposure model. Estimating the magnitude of this error is, however difficult, although the net effect would have been to reduce the power of the exposure-response analyses to detect a true exposure gradient if one existed.

Exposure to concentrate has, through monitoring urinary metabolites, been shown to be the principal route of exposure among those who handled it.[9] It was therefore decided to also analyse separately the effect of handling concentrate on the neurological responses. Cumulative events during which concentrate was handled were highly correlated with the other cumulative indices, but did not result in a significant improvement in fit for symptoms or the quantitative sensory test thresholds when compared with simple cumulative days dipped. However, among sheep dippers, average intensity of exposure to concentrate was strongly related to symptoms, independently of number of days dipped. This was largely determined by whether or not the sheep dipper had been a principal concentrate handler, which is an aspect of exposure history which might be expected to be recalled with reasonable accuracy.

The reliability of these findings may have been affected by non-participation of some selected subjects. However, the reasons for non-participation seemed plausibly not to be strongly related to health; and although some selection bias cannot be excluded, there is no positive indication that results have been seriously distorted, particularly given the strength of the effect of concentrate.

However, the ORs estimated in table 5 show that, after adjustment for age, country, and sex, the prevalence of symptoms among sheep dippers who were not concentrate handlers was lower than among farmers who were not sheep dippers, and the prevalence among dippers who did handle concentrate was roughly equal to this control group. This may reflect the difficulties of recruiting of pig and chicken farmers into a study about health of sheep dippers, where the principal benefit to participants was the chance of a free health examination. It is conceivable that farmers who were not sheep dippers, compared with sheep farmers, self selected to a greater degree on the basis of perceived symptoms.

The significant effects due to country of residence and sex were thought to have been due to reporting biases. As a check on possible differences in use of product between the two countries, the relative frequency of the names of OP sheep dip products recalled by sheep dippers in England and in Scotland were very similar. However, in a later clinical substudy, it was found that the prevalence of reporting symptoms among those Scottish farms was higher than in the field study and closer to the prevalence among English farmers (Pilkington *et al*, 1999).[18] This suggested that this effect was due to a measurement bias in the form of a reticence on the part of the Scottish farmers to report symptoms during the field survey.

Among the quantitative sensory test thresholds there was also evidence of trends relative to mean intensity of exposure to concentrate, although not as marked, and more difficult to interpret than for symptoms. The estimated peak effect, higher at moderate exposure intensities than at very low or very high intensities, was of strikingly similar shape across all three quantitative sensory test thresholds. A possible reason for a low effect at high intensities of exposure to concentrate was suggested by the evidence of greater use of protective gloves with increased frequency of handling concentrate. However, it is unclear why any possible protective effect should be more apparent for measured sensory thresholds than for symptoms.

All three quantitative sensory test measurements were positively associated with age, as would be expected, and this effect was generally independent of exposure. This provides some evidence that the quantitative sensory test measurements were reliable and the associated results were meaningful. The quantitative sensory test results also indicate, as for symptoms, that the principal evidence of a neurological effect is relative to exposure to concentrate.

Subsequently, it was decided to explore whether among sheep dippers, concentrate handlers (n=479) differed from non-handlers (n=133), and whether handling concentrate was acting as a surrogate for some other social or occupational factor which might influence the likelihood of reporting symptoms.

Job titles were broadly categorised according to whether the job title indicated management or ownership of the farm (83%); a farming role but excluding management role; principal area of work not farming. Current employer was categorised into those who indicated that the subject was self employed. Fifty seven per cent of non-handlers of concentrate were owners or managers compared with 73% of concentrate handlers. Indicators for each of the three occupational categories were included in the model for symptoms in table 5 in place of the (highly significant) effect of handling concentrate and the estimated ORs were 0.97, 1.34, and 1.50, respectively. Clearly, none of these occupational indicators is significantly associated with increased reporting of symptoms, particularly compared with handling concentrate (OR=3.4).

The mean age of concentrate handlers was 46 and non-handlers 43 years. Thirty eight per cent of handlers had O or A level certificates compared with 32% of non-handlers. Only for sex were there large differences between the two groups, 95% of handlers were male compared with 52% of non-handlers. However, sex and age were adjusted for in the earlier exposure-response analyses, and these confirmed that the effect of handling concentrate was apparent among both men and women. Therefore there was no strong evidence of a difference in occupational or social status between those classified as concentrate handlers and those not, sufficient to explain the magnitude of the effect of handling concentrate.

The original intention of the study was to examine the relations between exposure and a broad range of neurological symptoms. However, further models similar to that presented in table 5 were fitted looking at the effect of concentrate handling on the presence or absence of specific symptom groups: sensory, muscle weakness, and autonomic. These analyses showed that, although all types of symptoms were more commonly reported among concentrate handlers than non-handlers, the effect was most marked for sensory symptoms (OR=5.4) compared with either muscle weakness (OR=2.0) or autonomic (OR=2.2) symptoms, both of which were not significant. The sex and country effects noted earlier were broadly similar across the different symptom types.

This finding was supported by results from the follow on clinical study among a subgroup of dippers,[18] where neuropathy, when it was clinically diagnosed, was predominantly of the sensory type both symptomatically and neurophysiologically.

The weakness of the evidence for a cumulative effect, together with the strength of the evidence for an effect among concentrate handlers would not lend credence to the model of incremental neurological damage from low level exposure to OPs. However, it might be conjectured that repeated exposures to concentrated forms of OPs above a certain threshold, could be associated with long term health effects in a subgroup of people exposed without producing overt cholinergic effects.

Conclusions

The results showed higher rates of self reported symptoms among farmers generally compared with other workers, and evidence of higher cold sensation thresholds among sheep dippers exposed to OPs compared with non-exposed farmers. There was only limited evidence of a weak effect of low level cumulative exposure on reporting symptoms, and no evidence of such an effect on sensory thresholds.

The most important exposure factor was due to contact with concentrate dip, in that markedly higher rates of reported symptoms, adjusted for other factors, were reported among those who had at some time been principal concentrate handlers. There was also evidence for this effect of handling concentrate on measured sensory thresholds. These results suggest that long term health effects can result from exposure to higher concentrations of OPs within concentrate dip, but are less likely to occur from prolonged exposure to the diluted dip.

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